

**Amendment and Response**

Applicants: Michael S. KINCH et al.

Serial No.: 09/640,952

Filed: 17 August 2000

For: EPHA2 AS A DIAGNOSTIC TARGET FOR METASTATIC CANCER (As Amended)

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Amendments to the Claims

This listing of claims replaces all prior versions and listings of claims in the above-identified application:

1. (Currently amended) A method for detecting the presence of metastatic cells in a cell population comprising the steps of  
lysing at least a portion of the cell population,  
incubating the lysed cells with a monoclonal antibody that specifically binds EphA2 to allow antibody binding to EphA2, and  
detecting antibody-EphA2 binding; wherein antibody-EphA2 binding is indicative of the presence of metastatic cells in the cell population.
2. Canceled
3. (Currently amended) The method of ~~claim 2 wherein the epitope of EphA2 is~~ claim 1 wherein the antibody binds to an intracellular epitope of EphA2.
4. (Original) The method of claim 3 wherein the antibody is produced by hybridoma cell line D7.
5. (Currently amended) The method of claim ~~[[2]]~~ 1 wherein the antibody is labeled with a detectable label, and the detecting step includes detecting the label.
6. (Original) The method of claim 5 wherein the antibody is labeled with a fluorescent label and the detecting step comprises detecting the fluorescent label.

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7. (Original) The method of claim 5 wherein the antibody is labeled with a radioactive label and the detecting step comprises detecting the radioactive label.
8. (Original) The method of claim 1 wherein the cell population comprises cells from a breast or prostate tissue biopsy.
9. (Original) The method of claim 1 wherein the cell population is harvested from a body fluid selected from the group consisting of blood, plasma, spinal fluid, saliva, and urine.
10. (Original) The method of claim 9 wherein the detecting step includes a diagnostic method selected from the group consisting of ELISA assays and flow cytometry.
11. (Original) The method of claim 1 wherein the incubating and detecting steps comprise western blotting methodology.
12. (Original) The method of claim 11 further comprising the steps of  
providing a second antibody having phosphotyrosine specificity, and  
western blotting with the second antibody.
13. (Original) The method of claim 1 wherein the metastatic cells are selected from the group consisting of breast, prostate, lung, and colon cancers.
- 14.-20. Canceled
21. (Currently amended) A method for detecting the presence of metastatic cells in a cell ~~population~~ tissue sample comprising the steps of

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incubating the ~~cells~~ tissue sample with a reagent capable of specific binding to a nucleic acid coding for the EphA2 protein to allow reagent binding to the nucleic acid, and detecting ~~reagent-compound~~ reagent-nucleic acid binding; wherein reagent-nucleic acid binding is indicative of the presence of metastatic cells in the tissue sample.

22. Canceled

23. (Previously presented) The method of claim 21 wherein the nucleic acid is DNA or RNA.

24. (Original) The method of claim 21 further comprising the step of fixing the cells on a slide, and the detecting step comprises immunofluorescence staining.

25.-32. Canceled

33. (Currently amended) The method of claim 5 wherein the antibody comprises at least one of a fluorescent label, a chemiluminescent label, a bioluminescent label, an enzymatic label, a chromogenic label and a radiolabel, wherein detecting [reagent-EphA2] antibody-EphA2 binding comprises detecting at least one detectable label.

34. (Currently amended) The method of claim [[28]] 1 wherein the cell population comprises cells selected from the group consisting of breast cells, kidney cells, prostate cells, lung cells and colon cells.

35. (Currently amended) The method of claim [[28]] 1 wherein the cell population comprises epithelial cells.

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36. (Currently amended) The method of claim [[28]] 1 wherein the cell population comprises cells selected from the group consisting of breast cancer cells, kidney cancer cells, prostate cancer cells, lung cancer cells and colon cancer cells.

37. (Currently amended) The method of claim [[28]] 1 wherein the cell population comprises epithelial cancer cells.

38.-40. Canceled

41. (Currently amended) The method of claim [[28]] 1 wherein the cell population comprises cells from a tissue biopsy.

42. (Previously presented) The method of claim 41 wherein the tissue comprises breast tissue or prostate tissue.

43. (Currently amended) The method of claim [[28]] 1 wherein the cell population comprises cells from a body fluid.

44. (Previously presented) The method of claim 43 wherein the body fluid is selected from the group consisting of blood, plasma, spinal fluid, saliva, and urine.

45. (Currently amended) The method of claim [[28]] 1 wherein detecting antibody-EphA2 binding comprises utilizing a diagnostic method selected from the group consisting of an ELISA assay, a Western blot, and flow cytometry.

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46. (Currently amended) The method of claim [[28]] 1 wherein detecting antibody-EphA2 binding comprises utilizing a Western blot; the method further comprising Western blotting with a second antibody having phosphotyrosine specificity.

47. (Previously presented) A method for detecting the presence of metastatic cells in a cell population comprising:

incubating at least a portion of the cell population with a monoclonal antibody that specifically binds EphA2 to allow binding of the antibody to EphA2; and

detecting antibody-EphA2 binding, wherein antibody-EphA2 binding is indicative of the presence of metastatic cells in the cell population.

48. Canceled

49. (Previously presented) The method of claim 47 wherein the antibody binds to an intracellular epitope of EphA2.

50. (Previously presented) The method of claim 47 wherein the antibody is produced by hybridoma cell line D7.

51. (Previously presented) The method of claim 47 wherein the antibody binds to an extracellular epitope of EphA2.

52. (Previously presented) The method of claim of claim 47 wherein antibody-EphA2 binding yields a bound complex comprising a whole cell.

53. (Previously presented) The method of claim 52 wherein detecting antibody-EphA2 binding comprises subjecting the bound complex to immunohistochemical staining.

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54. (Previously presented) The method of claim 47 wherein the antibody is produced by hybridoma cell line B2D6.

55. (Previously presented) The method of claim 47 wherein the bound antibody comprises a detectable label; and wherein detecting antibody-EphA2 binding comprises detecting the label.

56. (Previously presented) The method of claim 47 wherein the bound antibody comprises at least one of a fluorescent label, a chemiluminescent label, a bioluminescent label, an enzymatic label, a chromogenic label and a radiolabel; and wherein detecting antibody-EphA2 binding comprises detecting at least one detectable label.

57. (Previously presented) The method of claim 47 wherein the cell population comprises cells selected from the group consisting of breast cells, kidney cells, prostate cells, lung cells and colon cells.

58. (Previously presented) The method of claim 47 wherein the cell population comprises epithelial cells.

59. (Previously presented) The method of claim 47 wherein the cell population comprises cells selected from the group consisting of breast cancer cells, kidney cancer cells, prostate cancer cells, lung cancer cells and colon cancer cells.

60. (Previously presented) The method of claim 47 wherein the cell population comprises epithelial cancer cells.

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61. (Previously presented) The method of claim 47 wherein the cell population comprises metastatic cancer cells.

62. (Previously presented) The method of claim 61 wherein the metastatic cells comprise cells selected from the group consisting of breast cancer cells, kidney cancer cells, prostate cancer cells, lung cancer cells, and colon cancer cells.

63. (Previously presented) The method of claim 47 wherein the metastatic cells comprise epithelial cancer cells.

64. (Previously presented) The method of claim 47 wherein the cell population comprises cells from a tissue biopsy

65. (Previously presented) The method of claim 64 wherein the tissue comprises breast tissue or prostate tissue.

66. (Previously presented) The method of claim 47 wherein the cell population comprises cells from a body fluid.

67. (Previously presented) The method of claim 66 wherein the body fluid is selected from the group consisting of blood, plasma, spinal fluid, saliva, and urine.

68. (Previously presented) The method of claim 47 wherein detecting reagent-EphA2 binding comprises utilizing a diagnostic method selected from the group consisting of an ELISA assay, a Western blot, and flow cytometry.

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69. (Previously presented) The method of claim 47 wherein detecting reagent-EphA2 binding comprises utilizing a Western blot; the method further comprising Western blotting with a second antibody having phosphotyrosine specificity.

70.-71. Canceled

72. (Previously presented) A method for detecting the presence of cancer cells in a selected cell population comprising:

assaying at least a portion of the selected cell population for at least one of  
a change in EphA2 intracellular localization pattern; and  
a change in EphA2 phosphorylation content

as compared to the intracellular localization pattern and phosphorylation content in an analogous normal cell population; wherein the change is indicative of the presence of a cancer cell in the selected cell population.

73. (Previously presented) The method of claim 72 wherein a change in intracellular localization pattern or phosphorylation content is indicative of the presence of metastatic cancer cells in the cell population.

74. Canceled.

75. (Previously presented) The method of claim 72 wherein assaying the cell population comprises incubating at least a portion of the selected cell population with a reagent capable of binding to EphA2 to allow binding of the reagent to EphA2; and detecting reagent-EphA2 binding.



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76. (Previously presented) The method of claim 75 wherein the reagent is an antibody.
77. (Previously presented) The method of claim 76 wherein the antibody is produced by hybridoma D7 or B2D6.
78. (Previously presented) A method for determining the disease stage in a cell population comprising cancer cells, the method comprising:
- assaying at least a portion of the cell population for at least one of
    - EphA2 intracellular localization; and
    - EphA2 phosphorylation content; and
  - determining the disease stage of the cancer cells.
79. (Previously presented) The method of claim 78 wherein assaying the cell population comprises incubating at least a portion of the cancer cell population with a reagent capable of binding to EphA2 to allow binding of the reagent to EphA2; and detecting reagent-EphA2 binding.
80. (Previously presented) The method of claim 79 wherein the reagent is an antibody.
81. (Previously presented) The method of claim 80 wherein the antibody is produced by hybridoma D7 or B2D6.
- 82.-89. Canceled
90. (Previously presented) A method for detecting the presence of cancer cells in a selected cell population comprising:
- assaying at least a portion of the selected cell population for at least one of

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a change in EphA2 expression level;

a change in EphA2 intracellular localization pattern; and

a change in EphA2 phosphorylation content

as compared to the EphA2 expression level, intracellular localization pattern and phosphorylation content in an analogous normal cell population;

wherein the assaying the cell population comprises incubating at least a portion of the selected cell population with a monoclonal antibody, and wherein the change is indicative of the presence of a cancer cell in the selected cell population.

91. (Currently amended) The method of claim [[82]] 90 wherein a change in EphA2 expression level is indicative of the presence of nonmetastatic cancer cells in the cell population.